# CHAPTER - 5

## EPIDERMAL WATER PERMEATION IN VERTEBRATES : AN *IN VITRO* STUDY USING TRITIATED WATER

The epidermis of the skin acts as a barrier for permeation of water and other hydrophilic substances. 'Permeation', is the penetration of a compound through one layer into another layer, which is both functionally and structurally different from the first layer (Schaefer et al., 1982).

The barrier to transepidermal water loss (TEWL) in mammals is formed by the extrusion of the lamellar body contents into the SC-SG (stratum corneum - stratum granulosum) interface. These lamellar polar lipids are converted to more nonpolar species, and are sequestered as lipid bilayers into the SC interstices (Elias et al., 1977; Elias, 1981).

Middleton (1968) suggested a possible mechanism of water binding in the SC. According to him, if the lipid bilayers are removed by solvent treatment, the barrier function of the skin is lost. This solvent treatment exposes the hygroscopic substances like proteins in the corneal cells, to the water solvent. The water binding ability of the SC is thus lost.

Later, Imokawa and Hattori (1985) put forward a possible role of the structural lipids in the water holding properties of the SC. Anything that is applied on the skin, thus has to pass through this lipid barrier.

Permeability of any molecule through the skin (in vivo) could be analysed by the disappearance of the radioactive isotope ($\beta$ emitters) applications on the skin. $\beta$ emitters are known to pass through the skin barrier and are thus considered to be effectively absorbed (Schaefer et al., 1982). Scanty information is available on the $\beta$ emitters used for such experiments.

To determine the rate of in vitro permeation of water through the epidermis, a 'permeability cell' was designed, where the epidermis was mounted on the permeability chamber and the diffusion of labelled water (tritiated water, $^3H_2O$) through this physical membrane was checked. The quantity of labelled water permeated was calculated by counting the radioactivity in the buffer that is placed under the epidermis (Scheuplein and Ross, 1974).

Similar procedure was used to ascess water permeation, using different samples of epidermis. This experiment was conducted to see if tritiated water could be used to study the water permeation through the skin (in vitro).

## MATERIALS AND METHOD

Neonatal rat pups (1 day old) were taken and the skin treated with :

1. 45% glycerol - 2 applications daily for 2 days.
2. 50% beewax in heptane - 2 applications daily for 2 days.
3. 70% Brij 99 [polyoxyethylene (10) oleyl ether] in water - 2 applications daily for 2 days.
4. Control : double distilled water - 2 applications daily for 2 days.

Quantity of the compounds applied were essentially the same i.e. 0.5 ml.

Whole thickness (i.e. epidermis + dermis) skin was removed after decapitation of the animals on the 3rd day. The epidermis was separated using heat splitting method (Kligman and Christophers, 1963; Polano et al.,

1972; van Scott, 1952). One of the control epidermal sheets was treated with solvent (chloroform : methanol, 2:1), prior to mounting on the permeability cell.

Pigeon skin (apterium) was similarly treated with 45% glycerol for 7 days. Control epidermis, glycerol treated and solvent treated epidermis were used for the permeability experiment.

Whole-thickness skin of lizard (dorsal and ventral) was mounted for the experiment, as the separation of the epidermal sheet was not possible.

Experimental Set Up : (Permeability Cell) : (Fig. 1)

A small glass cell of 5 ml capacity with both ends open was taken. Epidermal sheet was mounted on one side with the help of Krazy glue (cyanoacrylate glue). 1 ml of tritiated water (with $2.55 \times 10^6$ DPM/g ± 3% / 10 ml) diluted to 10 ml with physiological saline was used in the upper cell (2 ml each). This cell was then immersed in a lower cell containing phosphate buffer saline (PBS) (0.1 M, pH 7.2). The upper cell was immersed in such a way that the epidermal sheet just touched the surface of the buffer in the lower cell. 0.1 ml sample from the lower cell was taken at different time intervals of 10, 20, 30, 40, 50, 60, 80 and 120 minutes, respectively after shaking the solution in the lower cell thoroughly. 0.1 ml of fresh PBS was replaced after each time interval. The sample was taken in 10 ml scintillation fluid in scintillation vials. The radioactivity was counted on a LKB Rackbetta β Scintillation Counter. The scintillation fluid essentially consists of POPOP [p-di (2-(5-phenyloxazolyl) benzene] - 0.1 gms; PPO (2,5 diphenyl oxazole) - 5 gms; Naphthalene (scintillation grade) - 120 gms in one litre of dioxane.

Fig.1: Permeability cell

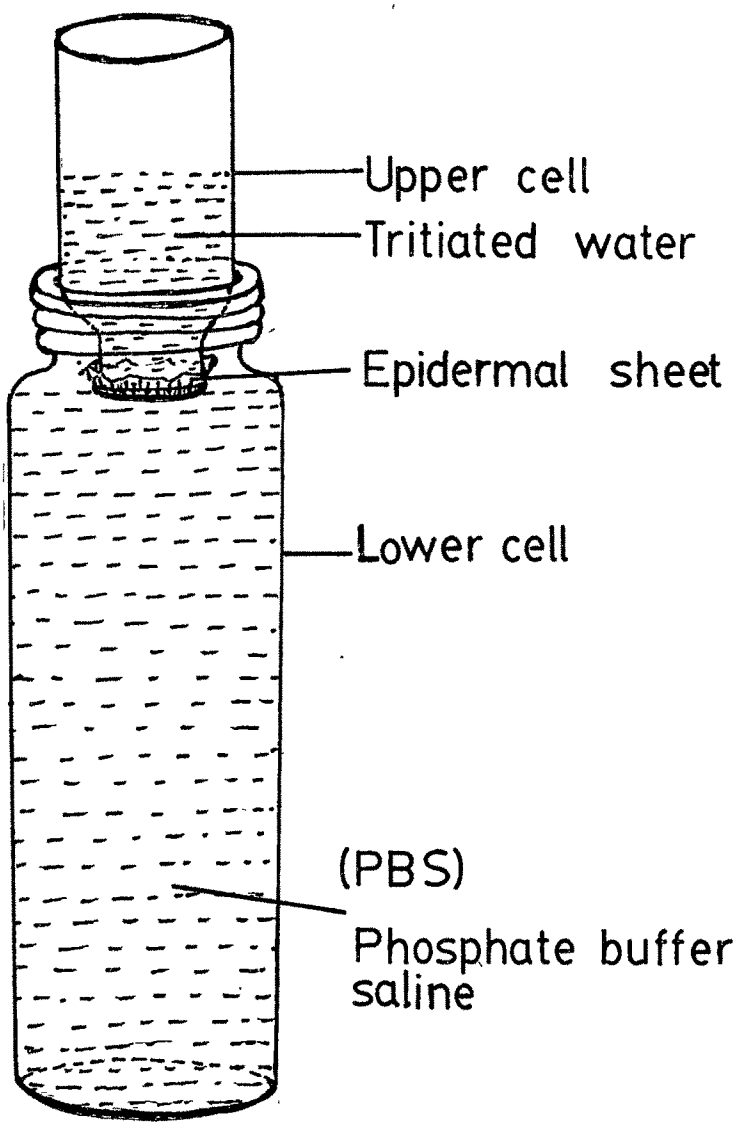

Table 1 : In vitro water permeation in epidermal sheets with different treatments in various animal models, using tritiated water ($^3H_2O$)*.

| Treatment (Sample) | CPM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time(mins): | 10 | 20 | 30 | 40 | 50 | 60 | 75 | 100 |
| Pigeon normal skin | 1291.42 | 1583.21 | 2049.41 | 2359.93 | 11053.12 | 13073.45 | 13490.48 | 13115.16 |
| Pigeon glycerine treated | 1297.03 | 1821.58 | 2018.49 | 2279.64 | 1951.17 | 1861.60 | 1836.34 | 2302.31 |
| Pigeon skin solvent treated | 2336.41 | 2306.48 | 2307.60 | 3146.85 | 2315.27 | 26187.91 | 35015.27 | 35115.17 |
| Normal rat pup skin | 1867.66 | 2386.91 | 1904.09 | 22994.02 | 17199.33 | 19132.66 | 22994.00 | 25019.70 |
| Rat pup skin - Glycerine treated | 1688.55 | 1714.23 | 2960.18 | 3154.87 | 3951.32 | 3834.49 | 3899.31 | 4734.03 |
| Rat pup skin - Beewax treated | 1574.73 | 1688.58 | 1914.96 | 1861.64 | 1729.31 | 2500.78 | 2688.59 | 2584.79 |
| Rat pup skin - Brij 99 treated | 1614.05 | 1713.53 | 2041.47 | 1616.03 | 2116.82 | 3214.69 | 3220.12 | 3028.05 |
| Rat pup skin - Solvent treated | 2206.81 | 2736.91 | 3426.09 | 3796.79 | 23146.85 | 26510.27 | 32687.91 | 35615.17 |
| Lizard whole skin - Ventral | 1704.47 | 3034.53 | 1517.68 | 1709.14 | 1776.86 | 1931.58 | 1973.52 | 1923.85 |
| Lizard whole skin - Dorsal | 2058.55 | 1926.65 | 2238.62 | 3225.92 | 3557.22 | 3995.51 | 3691.52 | 3510.48 |

* Radioactivity - ($2.55 \times 10^6$ DPM/g)

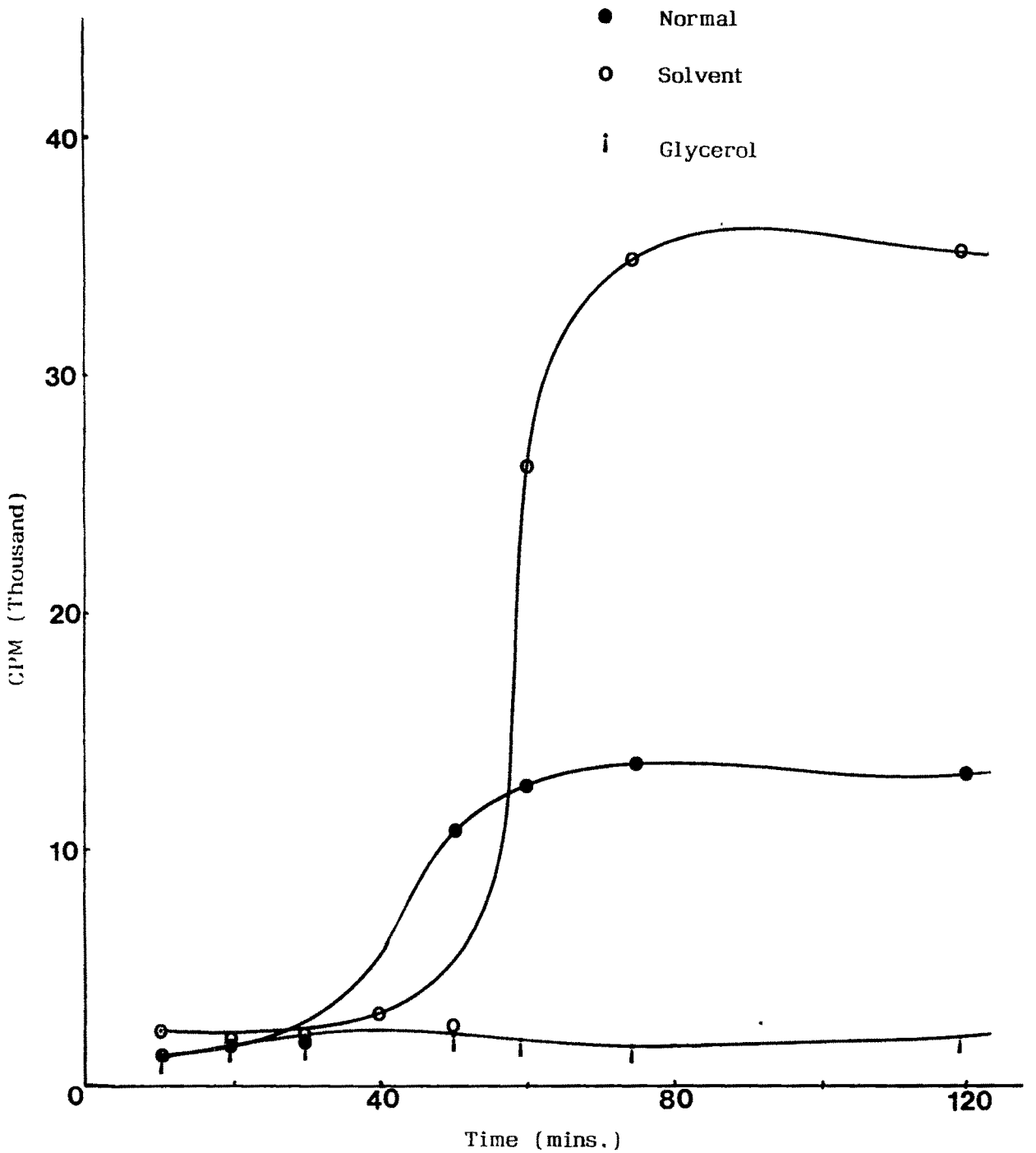


Fig. 1 : In vitro water permeation using pigeon epidermal sheets (apterium) after various treatments.

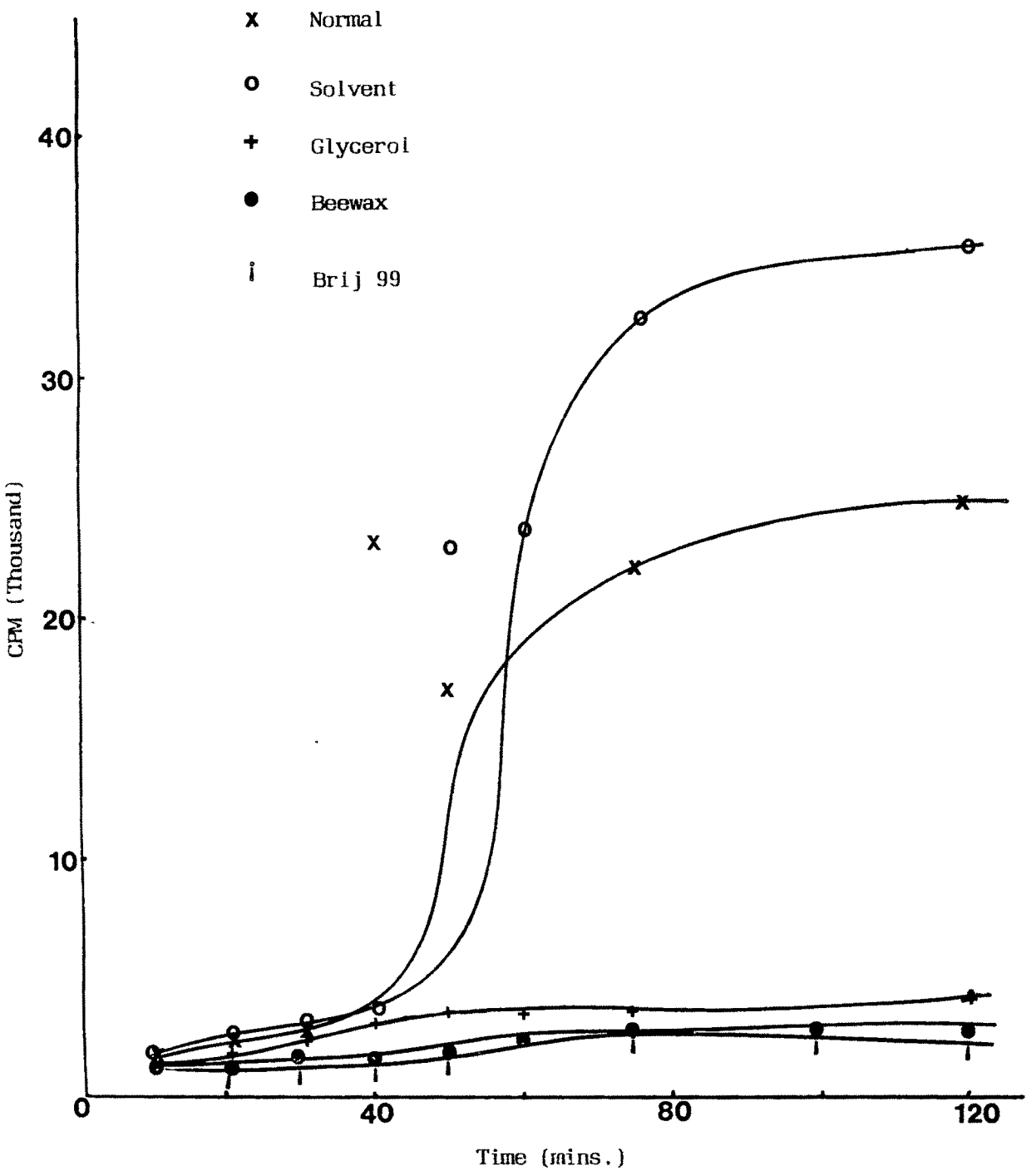


Fig. 2 : In vitro water permeation using rat pup epidermal sheets after various treatments.

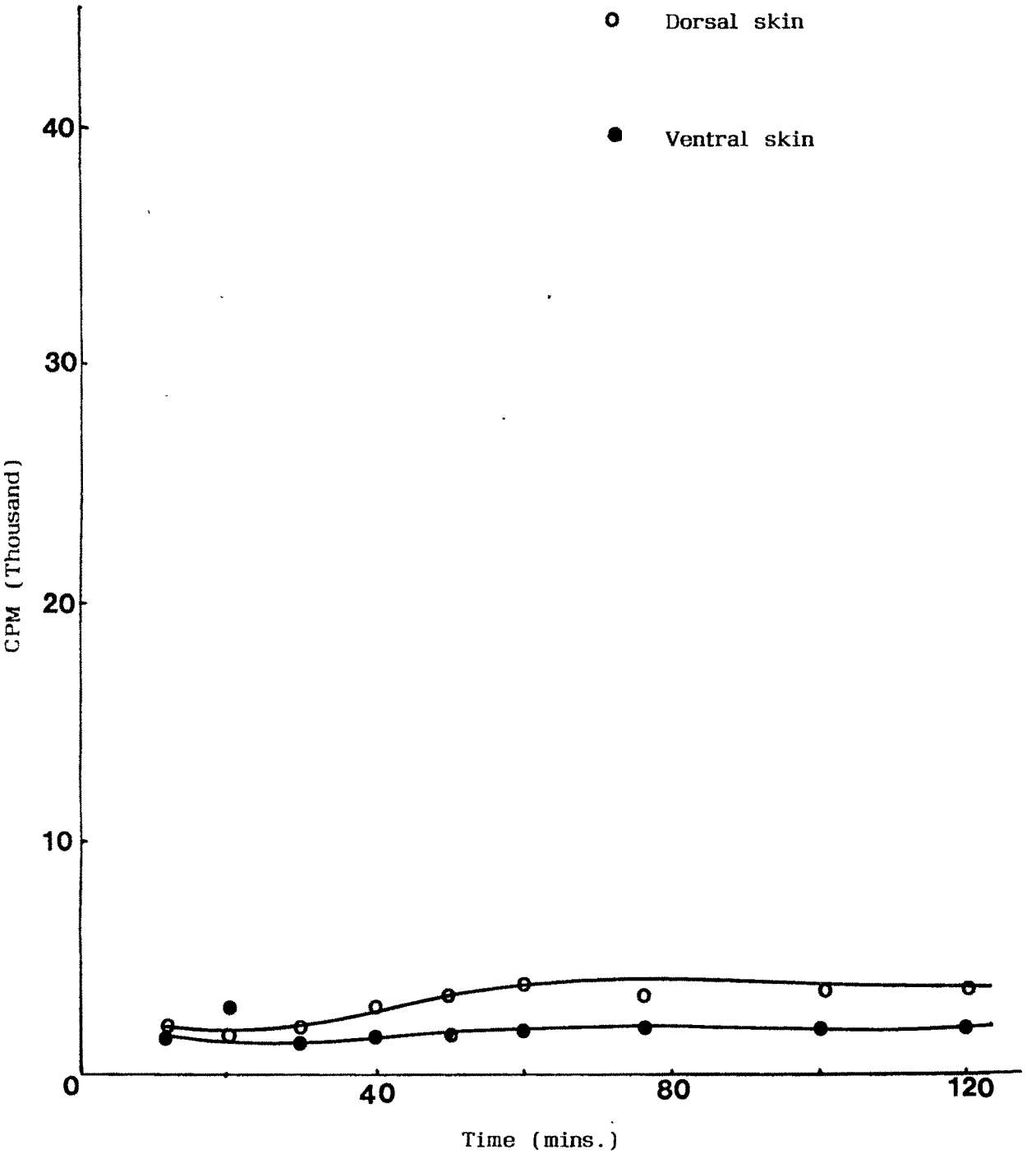


Fig. 3 : In vitro water permeation in lizard skin.

The graph of count versus time interval (minutes) was plotted.

## RESULTS AND DISCUSSION

Normal pigeon epidermis, shows a gradual increase in water permeation till 40 mins. Later, the tissue gets hydrated and there is an increase in permeation till 60 mins. The epidermis then shows negligible passage of water (Fig. 2).

Solvent treatment is known to remove all barrier lipids from the epidermis. These structural lipids hold water in SC (Imokawa and Hattori, 1985). When these lipids are removed, the epidermis initially shows a slight increase in water permeation, compared to control epidermis, till about 50 mins. After that, the epidermis acts like a sieve for the labelled water. There is an increase in permeation by 40-50%, which continues till 75 mins., after which the count becomes steady (Fig. 2).

Glycerine treatment of the skin leads to water retention in SC. Secondly it also helps in maintaining the liquid crystal structure of the intercellular lipid bilayers of the corneocytes (Froebe et al., 1990). This work supports the above data, as a significant decrease in water permeation (hardly 3%) is seen in the glycerine treated epidermis compared to the control / normal epidermis (Fig. 2).

Neonatal rat pup epidermis shows almost a similar trend of water permeation with glycerol and solvent treated epidermis compared to the normal epidermis. Glycerol treatment could thus help in decreasing TEWL (Fig. 3).

Brij 99, is used to prepare liquid crystalline creams, which act as a means for transdermal drug delivery system (Junginger et al., 1987). Beewax and Brij 99 were checked for their permeability effect, as both could be used as vehicles for topical applications of drugs on skin. Treatment of skin with both of these compounds, shows negligible permeation of water compared to the normal epidermis (Fig. 3). No references are available on the effect of these two vehicles on the transepidermal water loss. The present observations therefore, show that these two compounds could help in effective water retention in the epidermis.

Madderson et al. (1978) have shown that the reptilian epidermis is almost impermeable to water. These were further supported by Landmann (1979; 1980 a). Landmann et al. (1981), studied that permeation experiments with $LaNO_3$ (lanthanum nitrate) as a tracer in squamate reptiles indicated a strong barrier properties of the lipid lamellar sheets in the mesos layer to transcutaneous water flux. Observations with tritiated water further confirms that the lizard integument (both dorsal and ventral) allows very negligible loss of water, compared to both rat and pigeon epidermis (Fig. 4).

From the above studies, one could propose that tritiated water could be ideally used to determine in vitro water permeation through the skin.